XTO ENERGY

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Division of Water Quality
Wastewater Discharge Program

September 18, 2009

Alaska Dept. of Environmental Conservation
ATTN: Watershed Management Section
555 Cordova Street
Anchorage, Alaska 99501

FedEx Tracking Number: 7969 5804 3579

SUBJECT: DISCHARGE MONITORING REPORTS, NPDES PERMIT NUMBERS
AKG-31-5003 EAST FORELANDS FACILITY
AKG-31-5012 PLATFORM A
AKG-31-5013 PLATFORM C

Enclosed are the subject National Pollution Discharge Elimination System (NPDES) Discharge Monitoring Reports for the month of August 2009.

If there are any questions, please don't hesitate to contact me at (907) 776-2510 or Scott Griffith at (907) 776-2506.

Yours Truly,

Ryan Tunseth
Environmental, Health & Safety Coordinator

Enclosures: August 2009 DMR
2nd Period WET Test Report

cc: Director, Office of Water &Watersheds
U.S. Environmental Protection Agency
Region 10
1200 Sixth Avenue, OWW-130
Seattle, Washington 98101

Director, Office of Compliance and Enforcement
U.S. Environmental Protection Agency, Region 10
1200 Sixth Avenue, OCE-133
Seattle, Washington 98101

Scott Griffith
Mike Oconnor

NATIONAL POLLUTANT DISCHARGE ELIMINATION SYSTEM (NPDES)
DISCHARGE MONITORING REPORT (DMR)

NAME:	XTO ENERGY, INC
ADDRESS:	52260 WIK RD KENAI, AK 99611
FACILITY:	EAST FORELANDS
LOCATION:	60° 31' 10" N; 151° 20' 31" W

(2-16)	(17-19)
AKG 31 5003	015
PERMIT NUMBER	DISHCARGE NUMBER

☐ CHECK HERE IF NO DISCHARGE

MONITORING PERIOD

	YEAR	MONTH	DAY
FROM	2009	8	1
	(20-21)	(22-23)	(24-25)

	YEAR	MONTH	DAY
TO	2009	8	31
	(26-27)	(28-29)	(30-31)

PARAMETER (32-37)		QUANTITY OR LOADING (46-53) (54-61) Average	Maximum	Units	QUALITY OR CONCENTRATION (38-45) Minimum	(46-53) Average	(54-61) Maximum	Units	NO EX. (62-63)	FREQUENCY OF ANALYSIS (64-68)	SAMPLE TYPE (69-70)
015 - Produced Water FLOW	Sample Measurement	0.097656774	0.150528	MGD	***	***	***	***	0	Weekly	Estimate
	Permit Requirement	***	***		***	***	***		***	Weekly	Estimate
015 - Produced Water PRODUCED SAND	Sample Measurement	***	***	***	No discharge	No discharge	No discharge	***	0	***	***
	Permit Requirement	***	***		No discharge	No discharge	No discharge		***	***	***
015 - Produced Water OIL & GREASE	Sample Measurement	***	***	***	***	9.9	12	mg/l	0	Weekly	Grab
	Permit Requirement	***	***		***	29	42		***	Weekly	Grab
015 - Produced Water pH	Sample Measurement	***	***	***	7.06	***	7.4	SU	0	Weekly	Grab
	Permit Requirement	***	***		6	***	9		***	Weekly	Grab
015 - Produced Water TAH	Sample Measurement	***	***	***	***	19.18	19.18	mg/l	0	Monthly	Grab
	Permit Requirement	***	***		***	24	32		***	Monthly	Grab
015 - Produced Water TAqH	Sample Measurement	***	***	***	***	19.48	19.48	mg/l	0	Monthly	Grab
	Permit Requirement	***	***		***	Report	Report		***	Monthly	Grab
015 - Produced Water TOTAL AMMONIA	Sample Measurement	***	***	***	5.1	5.1	5.1	mg/l	0	Quarterly	Grab
	Permit Requirement	***	***		Report	***	Report		***	Quarterly	Grab

NAME TITLE PRINCIPAL EXECUTIVE OFFICER			Telephone	Date (YR/MO/DAY)
Ryan Tunseth HSE&T Coordinator	I certify under penalty of law that this document and all attachments were prepared under my direction or supervision in accordance with a system designed to assure that qualified personnel properly gather and evaluate the information submitted. Based on my inquiry of the person or persons who manage the system, or those persons driectly responsible for gathering the information, the information submitted is, to the best of my knowledge and belief is true, accurate, and complete. I am aware that there are significant penalties for submitting false information, including the possibility of fine and imprisonment for knowing violations.	Signature	907 776-2510	9/18/2009

COMMENTS & EXPLANATION OF ANY VIOLATIONS: WET Testing sampling frequency is reduced to once/6 months [Section II.G.6.a - Permit # AKG-31-5000] 2nd Period 2009 WET tests were taken on 07/21/09 and are submitted with this DMR. Additionally the sampling frequency for Copper, Manganese, Silver, Total Mercury, and Zinc is reduced from monthly to quarterly [Section II.G.6.a - Permit # AKG-31-5000] 3rd Quarter 2009 sample results are shown.

NATIONAL POLLUTANT DISCHARGE ELIMINATION SYSTEM (NPDES)
DISCHARGE MONITORING REPORT (DMR)

NAME:	XTO ENERGY, INC
ADDRESS:	52260 WIK RD KENAI, AK 99611
FACILITY:	EAST FORELANDS
LOCATION:	60° 31' 10" N; 151° 20' 31" W

(2-16)	(17-19)
AKG 31 5003	015
PERMIT NUMBER	DISHCARGE NUMBER

☐ CHECK HERE IF NO DISCHARGE

MONITORING PERIOD

	YEAR	MONTH	DAY
FROM	2009	8	1
	(20-21)	(22-23)	(24-25)

	YEAR	MONTH	DAY
TO	2009	8	31
	(26-27)	(28-29)	(30-31)

PARAMETER (32-37)		QUANTITY OR LOADING (46-53) (54-61) Average	Maximum	Units	QUALITY OR CONCENTRATION (38-45) Minimum	(46-53) Average	(54-61) Maximum	Units	NO EX. (62-63)	FREQUENCY OF ANALYSIS (64-68)	SAMPLE TYPE (69-70)
015 - Produced Water COPPER	Sample Measurement	***	***	***	***	3.82	3.82	ug/l	0	Quarterly	Grab
	Permit Requirement	***	***		***	60	90		***	Quarterly	Grab
015 - Produced Water MERCURY	Sample Measurement	***	***	***	***	N/D	N/D	ug/l	0	Quarterly	Grab
	Permit Requirement	***	***		***	0.5	0.8		***	Quarterly	Grab
015 - Produced Water MANGANESE	Sample Measurement	***	***	***	***	1.04	1.04	mg/l	0	Quarterly	Grab
	Permit Requirement	***	***		***	7.9	15.8		***	Quarterly	Grab
015 - Produced Water SILVER	Sample Measurement	***	***	***	***	1.87	1.87	ug/l	0	Quarterly	Grab
	Permit Requirement	***	***		***	46	149		***	Quarterly	Grab
015 - Produced Water ZINC	Sample Measurement	***	***	***	***	0.342	0.342	mg/l	0	Quarterly	Grab
	Permit Requirement	***	***		***	3.1	6.1		***	Quarterly	Grab
015 - Produced Water WET - *Mytilus galloprovincialis* (invertibrate)	Sample Measurement	***	***	***	***	< 625	< 625	TUc	0	Semi Annual	Grab
	Permit Requirement	***	***		***	1209	2425		***	Semi Annual	Grab

NAME TITLE PRINCIPAL EXECUTIVE OFFICER			Telephone	Date (YR/MO/DAY)
Ryan Tunseth HSE&T Coordinator	I certify under penalty of law that this document and all attachments were prepared under my direction or supervision in accordance with a system designed to assure that qualified personnel properly gather and evaluate the information submitted. Based on my inquiry of the person or persons who manage the system, or those persons driectly responsible for gathering the information, the information submitted is, to the best of my knowledge and belief is true, accurate, and complete. I am aware that there are significant penalties for submitting false information, including the possibility of fine and imprisonment for knowing violations.	Signature	907 776-2510	9/18/2009

COMMENTS & EXPLANATION OF ANY VIOLATIONS: WET Testing sampling frequency is reduced to once/6 months [Section II.G.6.a - Permit # AKG-31-5000] 2nd Period 2009 WET tests were taken on 07/21/09 and are submitted with this DMR. Additionally the sampling frequency for Copper, Manganese, Silver, Total Mercury, and Zinc is reduced from monthly to quarterly [Section II.G.6.a - Permit # AKG-31-5000] 3rd Quarter 2009 sample results are shown.

NATIONAL POLLUTANT DISCHARGE ELIMINATION SYSTEM (NPDES)
DISCHARGE MONITORING REPORT (DMR)

NAME:	XTO ENERGY, INC
ADDRESS:	52260 WIK RD KENAI, AK 99611
FACILITY:	EAST FORELANDS
LOCATION:	60° 31' 10" N; 151° 20' 31" W

(2-16)	(17-19)
AKG 31 5003	015
PERMIT NUMBER	DISHCARGE NUMBER

[ ] CHECK HERE IF NO DISCHARGE

MONITORING PERIOD

	YEAR	MONTH	DAY
FROM	2009	8	1
	(20-21)	(22-23)	(24-25)
TO	2009	8	31
	(26-27)	(28-29)	(30-31)

PARAMETER (32-37)		QUANTITY OR LOADING (46-53) Average	(54-61) Maximum	Units	QUALITY OR CONCENTRATION (38-45) Minimum	(46-53) Average	(54-61) Maximum	Units	NO EX. (62-63)	FREQUENCY OF ANALYSIS (64-68)	SAMPLE TYPE (69-70)
015 - Produced Water WET -	Sample Measurement	***	***	***	***	***	***	TUc	0	Semi Annual	Grab
	Permit Requirement	***	***		***	1209	2425		***	Semi Annual	Grab
015 - Produced Water	Sample Measurement										
	Permit Requirement										
015 - Produced Water	Sample Measurement										
	Permit Requirement										
015 - Produced Water	Sample Measurement										
	Permit Requirement										
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	Permit Requirement										
015 - Produced Water	Sample Measurement										
	Permit Requirement										
015 - Produced Water	Sample Measurement										
	Permit Requirement										

NAME TITLE PRINCIPAL EXECUTIVE OFFICER			Telephone	Date (YR/MO/DAY)
Ryan Tunseth HSE&T Coordinator	I certify under penalty of law that this document and all attachments were prepared under my direction or supervision in accordance with a system designed to assure that qualified personnel properly gather and evaluate the information submitted. Based on my inquiry of the person or persons who manage the system, or those persons directly responsible for gathering the information, the information submitted is, to the best of my knowledge and belief is true, accurate, and complete. I am aware that there are significant penalties for submitting false information, including the possibility of fine and imprisonment for knowing violations.	Signature	907 776-2510	9/18/2009

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TOXICITY TEST REPORT

TEST IDENTIFICATION

Test No.: 663-61

Title: Mussel (*Mytilus galloprovincialis*) larval test using static 48-hr exposure to XTO Energy – East Foreland.

Protocol No.: NAS-XXX-CG/MG2, August 28, 1990, Revision 3 (9-8-01). This protocol complies with the U.S. EPA West Coast chronic toxicity manual (EPA/600/R-95/136) and ASTM bivalve toxicity method (E 724-89).

STUDY MANAGEMENT

Study Sponsor: XTO Energy, 52260 Wik Rd, Kenai, AK 99611

Sponsor's Study Monitor: Mr. Ryan Tunseth

Testing Laboratory: Northwestern Aquatic Sciences, P.O. Box 1437, Newport, OR 97365.

Test Location: Newport laboratory.

Laboratory's Study Personnel: G.A. Buhler, B.S., Proj. Man.; G.J. Irissarri, B.S., Study Dir.; L.K. Nemeth, B.A., M.B.A., QA Officer; M.S. Redmond, M.S., Aq. Toxicol.; S.J. Gage, B.A., Sr. Tech.

Study Schedule:

Test Beginning: 7-22-09, 1445 hrs.

Test Ending: 7-24-09, 1450 hrs.

Disposition of Study Records: All specimens, raw data, reports and other study records are stored according to Good Laboratory Practice regulations at Northwestern Aquatic Sciences, 3814 Yaquina Bay Rd., Newport, OR 97365.

Good Laboratory Practices: The test was conducted following the principles of Good Laboratory Practices (GLP) as defined in the EPA/TSCA Good Laboratory Practice regulations revised August 17, 1989 (40 CFR Part 792).

Statement of Quality Assurance: The test data were reviewed by the Quality Assurance Unit to assure that the study was performed in accordance with the protocol and standard operating procedures. This report is an accurate reflection of the raw data.

TEST MATERIAL

Description: XTO Energy East Foreland. Details are as follows:

NAS Sample No.	2797G
Collection Date	7-21-09
Receipt Date	7-22-09
Temperature (°C)	4.4
pH	7.9
Dissolved oxygen (mg/L)	0.9
Salinity (‰)	22.5

Treatments: Sample was briefly temperature-equilibrated prior to use.

Storage: Used date of receipt.

DILUTION WATER

Source: Yaquina Bay, Oregon.

Date of Collection: 7-21-09

Water Quality: Salinity, 30.0 ‰; pH, 8.1

Pretreatment: Filtered to 0.4 µm, aerated, salinity adjusted with Milli-Q water.

BRINE USED FOR SALINITY CONTROL

None Used

TEST ORGANISMS

Species: Mussel (*Mytilus galloprovincialis*).
Age: 2.0 hours post-fertilization.
Source: Carlsbad Aquafarm, Carlsbad, CA.
Conditioning: Adult mussels were received on 7-17-09 and placed in trays with flowing seawater. Holding conditions for the five days prior to the test averaged: temperature, 16.8 ± 1.4°C; pH, 7.3 ± 0.6; salinity, 33.8 ± 0.3 ‰; and dissolved oxygen, 5.6 ± 0.8 mg/L. Photoperiod was natural daylight.
Source of Gametes: 1 female and 1 male.

TEST PROCEDURES AND CONDITIONS

Test Chambers: 30 ml borosilicate glass vials containing 10 ml of test solutions.
Test Concentrations: 0.16, 0.08, 0.04, 0.02, 0.01, and 0% (Control).
Brine Control: None used
Replicates/Treatment: 4
Initial Concentration of Test Organisms: 27.2/ml.
Volume of Subsamples Taken for Counting: NA
Water Volume Changes per 24 hr: None (non-renewal static test).
Aeration: None
Feeding: None
Effects Criteria: The effect criteria used were: 1) ability of embryos to survive and produce completely developed shells; and 2) survival. Data collected were: 1) the initial embryo density; 2) the number of abnormal larvae observed; and 3) the number of normal (live with completely developed shells) larvae observed.
Water Quality and Other Test Conditions: Temperature, 15.6 ± 0.3°C; pH, 8.2 ± 0.1; salinity, 29.9 ± 0.2 ‰; and dissolved oxygen, 8.1 ± 0.1 mg/L. Photoperiod 16:8 hr, L:D.

DATA ANALYSIS METHODS

The proportion of surviving larvae, and the proportion of normal surviving larvae were calculated for each treatment replicate. The calculation used for the proportion of normal surviving larvae, Combined Proportion Normal, was the combined endpoint specified by EPA/600/R-95/136. The means were obtained for each treatment level and the latter were then corrected for control response using Abbott's formula. The LC50 (survival) and the EC50 (normality) were calculated, where data permitted, using either the Maximum-Likelihood Probit or the Trimmed Spearman-Karber methods. An IC25 was determined by linear interpolation with bootstrapping. NOEC and LOEC values for survival and normality were computed using either Dunnett's test, T-test with Bonferroni's adjustment, Steel's Many-One Rank Test, or Wilcoxon Rank Sum Test with Bonferroni Adjustment. The appropriate test was selected after evaluating the data for normality and homogeneity of variance. An arcsine-square root (angular) transformation was performed on the data prior to statistical analysis. The statistical software employed for these calculations was CETIS, v1.7.0C, Tidepool Scientific Software. Toxic units (TU_c) were computed as 100/NOEC, 100/EC50, or 100/IC25.

PROTOCOL DEVIATIONS

None

REFERENCE TOXICANT TEST

The routine reference toxicant test is a standard multi-concentration toxicity test using copper sulfate to evaluate the performance of the test organisms used in the effluent toxicity test. The performance is evaluated by comparing the results of this test with historical results obtained at the laboratory. A summary of the reference toxicant test result is given below. The reference toxicant test raw data are found in Appendix III.

Test No.: 999-2595
Reference Toxicant and Source: Copper as $CuSO_4 \cdot 5H_2O$, Argent Lot No. 0195. Concentrated stock prepared 8-3-07.
Test Date: 7-22-09
Dilution Water Used: Yaquina Bay, OR seawater. Salinity 30.0 ‰, pH 8.1.

Results: EC50, 10.9 µg/L; NOEC, 8 µg/L; IC25, 9.42 µg/L. The EC50 results are within the laboratory's control chart warning limits (8.02 – 12.1 µg/L).

TEST RESULTS

Detailed tabulations of the test results are given in Table 1. The biological effects, given as the NOEC, LOEC, EC50/LC50 for normality and survival, and IC25 for normality are summarized below.

	Combined Proportion Normal	Survival
NOEC (%)	0.16 (TU_c=625)	0.16 (TU_c=625)
LOEC (%)	>0.16 (TU_c<625)	>0.16 (TU_c<625)
EC50/LC50 (%)	>0.16 (TU_c<625)	>0.16 (TU_c<625)
(95% C.I.)	---	---
Method of Calculation	By Data Inspection	By Data Inspection
IC25 (%)	>0.16 (TU_c<625)	
(95% C.I.)	---	
Method of Calculation	Linear Interpolation	

DISCUSSION/CONCLUSIONS

The NOEC was 0.16 % effluent, and the EC50 and IC25 for abnormal development were both >0.16 %.

STUDY APPROVAL

Project Manager — Date: 8-21-09

Study Director — Date: 8-21-09

Laboratory Director — Date: 8/21/09

Quality Assurance Unit — Date: 8/19/09

Table 1. Test response of mussel (*Mytilus galloprovincialis*) larvae exposed to XTO Energy – East Foreland.

Test Material					Combined Proportion Normal*		Proportion Survived*	
Concentration (%)	Repl.	Norm.	Abn.	Total		Mean		Mean
0.16	1	253	7	260	0.930		0.956	
	2	212	6	218	0.779		0.802	
	3	260	10	270	0.956		0.993	
	4	245	3	248	0.901	0.892	0.912	0.915
0.08	1	234	8	242	0.860		0.890	
	2	248	9	257	0.912		0.945	
	3	242	6	248	0.890		0.912	
	4	221	7	228	0.813	0.869	0.838	0.896
0.04	1	270	6	276	0.993		1.000	
	2	251	3	254	0.923		0.934	
	3	247	6	253	0.908		0.930	
	4	234	5	239	0.860	0.921	0.879	0.936
0.02	1	235	7	242	0.864		0.890	
	2	230	6	236	0.846		0.868	
	3	268	4	272	0.985		1.000	
	4	252	9	261	0.927	0.905	0.960	0.929
0.01	1	274	5	279	0.982		1.000	
	2	263	8	271	0.967		0.996	
	3	249	5	254	0.915		0.934	
	4	238	3	241	0.875	0.935	0.886	0.954
Normal Control	1	231	9	240	0.849		0.882	
	2	252	3	255	0.927		0.938	
	3	225	5	230	0.827		0.846	
	4	247	3	250	0.908	0.878	0.919	0.896

* Based on an average initial count of 272 embryos per 10 ml sample, except that for the case in the combined proportion normal endpoint where number normal>average initial count, number normal is divided by the total count (as per EPA/600/R-95/136).

† Result significantly different (P≤0.05) from the control.

TOXICITY TEST REPORT

TEST IDENTIFICATION

Test No.: 663-63

Title: Mussel (*Mytilus galloprovincialis*) larval test using static 48-hr exposure to XTO Energy – Platform A – non-contact cooling water.

Protocol No.: NAS-XXX-CG/MG2, August 28, 1990, Revision 3 (9-8-01). This protocol complies with the U.S. EPA West Coast chronic toxicity manual (EPA/600/R-95/136) and ASTM bivalve toxicity method (E 724-89).

STUDY MANAGEMENT

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Sponsor's Study Monitor: Mr. Ryan Tunseth

Testing Laboratory: Northwestern Aquatic Sciences, P.O. Box 1437, Newport, OR 97365.

Test Location: Newport laboratory.

Laboratory's Study Personnel: G.A. Buhler, B.S., Proj. Man.; G.J. Irissarri, B.S., Study Dir.; L.K. Nemeth, B.A., M.B.A., QA Officer; M.S. Redmond, M.S., Aq. Toxicol.; S.J. Gage, B.A., Sr. Tech.

Study Schedule:

Test Beginning: 7-22-09, 1445 hrs.

Test Ending: 7-24-09, 1450 hrs.

Disposition of Study Records: All specimens, raw data, reports and other study records are stored according to Good Laboratory Practice regulations at Northwestern Aquatic Sciences, 3814 Yaquina Bay Rd., Newport, OR 97365.

Good Laboratory Practices: The test was conducted following the principles of Good Laboratory Practices (GLP) as defined in the EPA/TSCA Good Laboratory Practice regulations revised August 17, 1989 (40 CFR Part 792).

Statement of Quality Assurance: The test data were reviewed by the Quality Assurance Unit to assure that the study was performed in accordance with the protocol and standard operating procedures. This report is an accurate reflection of the raw data.

TEST MATERIAL

Description: XTO Energy – Platform A – non-contact cooling water. Details are as follows:

NAS Sample No.	2799G
Collection Date	7-21-09
Receipt Date	7-22-09
Temperature (°C)	5.1
pH	8.1
Dissolved oxygen (mg/L)	10.6
Salinity (‰)	26.0

Treatments: Sample was briefly temperature-equilibrated prior to use.

Storage: Used date of receipt.

DILUTION WATER

Source: Yaquina Bay, Oregon.

Date of Collection: 7-21-09

Water Quality: Salinity, 30.0 ‰; pH, 8.1

Pretreatment: Filtered to 0.4 µm, aerated, salinity adjusted with Milli-Q water.

BRINE USED FOR SALINITY CONTROL

None Used

TEST ORGANISMS

Species: Mussel (*Mytilus galloprovincialis*).
Age: 2.0 hours post-fertilization.
Source: Carlsbad Aquafarm, Carlsbad, CA.
Conditioning: Adult mussels were received on 7-17-09 and placed in trays with flowing seawater. Holding conditions for the five days prior to the test averaged: temperature, 16.8 ± 1.4°C; pH, 7.3 ± 0.6; salinity, 33.8 ± 0.3 ‰; and dissolved oxygen, 5.6 ± 0.8 mg/L. Photoperiod was natural daylight.
Source of Gametes: 1 female and 1 male.

TEST PROCEDURES AND CONDITIONS

Test Chambers: 30 ml borosilicate glass vials containing 10 ml of test solutions.
Test Concentrations: 0.16, 0.08, 0.04, 0.02, 0.01, and 0% (Control).
Brine Control: None used
Replicates/Treatment: 4
Initial Concentration of Test Organisms: 27.2/ml.
Volume of Subsamples Taken for Counting: NA
Water Volume Changes per 24 hr: None (non-renewal static test).
Aeration: None
Feeding: None
Effects Criteria: The effect criteria used were: 1) ability of embryos to survive and produce completely developed shells; and 2) survival. Data collected were: 1) the initial embryo density; 2) the number of abnormal larvae observed; and 3) the number of normal (live with completely developed shells) larvae observed.
Water Quality and Other Test Conditions: Temperature, 15.5 ± 0.4°C; pH, 8.1 ± 0.0; salinity, 29.9 ± 0.2 ‰; and dissolved oxygen, 8.1 ± 0.1 mg/L. Photoperiod 16:8 hr, L:D.

DATA ANALYSIS METHODS

The proportion of surviving larvae, and the proportion of normal surviving larvae were calculated for each treatment replicate. The calculation used for the proportion of normal surviving larvae, Combined Proportion Normal, was the combined endpoint specified by EPA/600/R-95/136. The means were obtained for each treatment level and the latter were then corrected for control response using Abbott's formula. The LC50 (survival) and the EC50 (normality) were calculated, where data permitted, using either the Maximum-Likelihood Probit or the Trimmed Spearman-Karber methods. An IC25 was determined by linear interpolation with bootstrapping. NOEC and LOEC values for survival and normality were computed using either Dunnett's test, T-test with Bonferroni's adjustment, Steel's Many-One Rank Test, or Wilcoxon Rank Sum Test with Bonferroni Adjustment. The appropriate test was selected after evaluating the data for normality and homogeneity of variance. An arcsine-square root (angular) transformation was performed on the data prior to statistical analysis. The statistical software employed for these calculations was CETIS, v1.7.0C, Tidepool Scientific Software. Toxic units (TU_c) were computed as 100/NOEC, 100/EC50, or 100/IC25.

PROTOCOL DEVIATIONS

None

REFERENCE TOXICANT TEST

The routine reference toxicant test is a standard multi-concentration toxicity test using copper sulfate to evaluate the performance of the test organisms used in the effluent toxicity test. The performance is evaluated by comparing the results of this test with historical results obtained at the laboratory. A summary of the reference toxicant test result is given below. The reference toxicant test raw data are found in Appendix III.

Test No.: 999-2595
Reference Toxicant and Source: Copper as $CuSO_4 \cdot 5H_2O$, Argent Lot No. 0195. Concentrated stock prepared 8-3-07.
Test Date: 7-22-09
Dilution Water Used: Yaquina Bay, OR seawater. Salinity 30.0 ‰, pH 8.1.

Results: EC50, 10.9 µg/L; NOEC, 8 µg/L; IC25, 9.42 µg/L. The EC50 results are within the laboratory's control chart warning limits (8.02 – 12.1 µg/L).

TEST RESULTS

Detailed tabulations of the test results are given in Table 1. The biological effects, given as the NOEC, LOEC, EC50/LC50 for normality and survival, and IC25 for normality are summarized below.

	Combined Proportion Normal	Survival
NOEC (%)	0.16 (TU_c=625)	0.16 (TU_c=625)
LOEC (%)	>0.16 (TU_c<625)	>0.16 (TU_c<625)
EC50/LC50 (%)	>0.16 (TU_c<625)	>0.16 (TU_c<625)
(95% C.I.)	—	—
Method of Calculation	By Data Inspection	By Data Inspection
IC25 (%)	>0.16 (TU_c<625)	
(95% C.I.)	—	
Method of Calculation	Linear Interpolation	

DISCUSSION/CONCLUSIONS

The NOEC was 0.16 % effluent, and the EC50 and IC25 for abnormal development were both >0.16 %.

STUDY APPROVAL

Project Manager — Date 8-21-09

Study Director — Date 8-21-09

Laboratory Director — Date 8/21/09

Quality Assurance Unit — Date 8/19/09

Table 1. Test response of mussel (*Mytilus galloprovincialis*) larvae exposed to XTO Energy – Platform A – non-contact cooling water.

Test Material					Combined Proportion Normal*		Proportion Survived*	
Concentration (%)	Repl.	Norm.	Abn.	Total		Mean		Mean
0.16	1	260	4	264	0.956		0.971	
	2	238	2	240	0.875		0.882	
	3	241	7	248	0.886		0.912	
	4	270	7	277	0.993	0.927	1.000	0.941
0.08	1	250	5	255	0.919		0.938	
	2	261	9	270	0.960		0.993	
	3	240	5	245	0.882		0.901	
	4	274	6	280	0.979	0.935	1.000	0.958
0.04	1	260	5	265	0.956		0.974	
	2	241	2	243	0.886		0.893	
	3	257	4	261	0.945		0.960	
	4	266	11	277	0.978	0.941	1.000	0.957
0.02	1	248	9	257	0.912		0.945	
	2	229	2	231	0.842		0.849	
	3	224	6	230	0.824		0.846	
	4	244	6	250	0.897	0.869	0.919	0.890
0.01	1	265	7	272	0.974		1.000	
	2	242	6	248	0.890		0.912	
	3	253	8	261	0.930		0.960	
	4	228	6	234	0.838	0.908	0.860	0.933
Normal Control	1	269	5	274	0.989		1.000	
	2	270	3	273	0.993		1.000	
	3	256	6	262	0.941		0.963	
	4	246	4	250	0.904	0.957	0.919	0.971

* Based on an average initial count of 272 embryos per 10 ml sample, except that for the case in the combined proportion normal endpoint where number normal>average initial count, number normal is divided by the total count (as per EPA/600/R-95/136).

† Result significantly different (P≤0.05) from the control.

TOXICITY TEST REPORT

TEST IDENTIFICATION

Test No.: 663-62

Title: Mussel (*Mytilus galloprovincialis*) larval test using static 48-hr exposure to XTO Energy – Platform A – water flood.

Protocol No.: NAS-XXX-CG/MG2, August 28, 1990, Revision 3 (9-8-01). This protocol complies with the U.S. EPA West Coast chronic toxicity manual (EPA/600/R-95/136) and ASTM bivalve toxicity method (E 724-89).

STUDY MANAGEMENT

Study Sponsor: XTO Energy, 52260 Wik Rd, Kenai, AK 99611

Sponsor's Study Monitor: Mr. Ryan Tunseth

Testing Laboratory: Northwestern Aquatic Sciences, P.O. Box 1437, Newport, OR 97365.

Test Location: Newport laboratory.

Laboratory's Study Personnel: G.A. Buhler, B.S., Proj. Man.; G.J. Irissarri, B.S., Study Dir.; L.K. Nemeth, B.A., M.B.A., QA Officer; M.S. Redmond, M.S., Aq. Toxicol.; S.J. Gage, B.A., Sr. Tech.

Study Schedule:

Test Beginning: 7-22-09, 1445 hrs.

Test Ending: 7-24-09, 1450 hrs.

Disposition of Study Records: All specimens, raw data, reports and other study records are stored according to Good Laboratory Practice regulations at Northwestern Aquatic Sciences, 3814 Yaquina Bay Rd., Newport, OR 97365.

Good Laboratory Practices: The test was conducted following the principles of Good Laboratory Practices (GLP) as defined in the EPA/TSCA Good Laboratory Practice regulations revised August 17, 1989 (40 CFR Part 792).

Statement of Quality Assurance: The test data were reviewed by the Quality Assurance Unit to assure that the study was performed in accordance with the protocol and standard operating procedures. This report is an accurate reflection of the raw data.

TEST MATERIAL

Description: XTO Energy – Platform A – water flood. Details are as follows:

NAS Sample No.	2798G
Collection Date	7-21-09
Receipt Date	7-22-09
Temperature (°C)	4.7
pH	8.1
Dissolved oxygen (mg/L)	10.4
Salinity (‰)	26.0

Treatments: Sample was briefly temperature-equilibrated prior to use.

Storage: Used date of receipt.

DILUTION WATER

Source: Yaquina Bay, Oregon.

Date of Collection: 7-21-09

Water Quality: Salinity, 30.0 ‰; pH, 8.1

Pretreatment: Filtered to 0.4 µm, aerated, salinity adjusted with Milli-Q water.

BRINE USED FOR SALINITY CONTROL

None Used

TEST ORGANISMS

Species: Mussel (*Mytilus galloprovincialis*).
Age: 2.0 hours post-fertilization.
Source: Carlsbad Aquafarm, Carlsbad, CA.
Conditioning: Adult mussels were received on 7-17-09 and placed in trays with flowing seawater. Holding conditions for the five days prior to the test averaged: temperature, 16.8 ± 1.4°C; pH, 7.3 ± 0.6; salinity, 33.8 ± 0.3 ‰; and dissolved oxygen, 5.6 ± 0.8 mg/L. Photoperiod was natural daylight.
Source of Gametes: 1 female and 1 male.

TEST PROCEDURES AND CONDITIONS

Test Chambers: 30 ml borosilicate glass vials containing 10 ml of test solutions.
Test Concentrations: 0.16, 0.08, 0.04, 0.02, 0.01, and 0% (Control).
Brine Control: None used
Replicates/Treatment: 4
Initial Concentration of Test Organisms: 27.2/ml.
Volume of Subsamples Taken for Counting: NA
Water Volume Changes per 24 hr: None (non-renewal static test).
Aeration: None
Feeding: None
Effects Criteria: The effect criteria used were: 1) ability of embryos to survive and produce completely developed shells; and 2) survival. Data collected were: 1) the initial embryo density; 2) the number of abnormal larvae observed; and 3) the number of normal (live with completely developed shells) larvae observed.
Water Quality and Other Test Conditions: Temperature, 15.6 ± 0.3°C; pH, 8.2 ± 0.1; salinity, 29.8 ± 0.2 ‰; and dissolved oxygen, 8.1 ± 0.1 mg/L. Photoperiod 16:8 hr, L:D.

DATA ANALYSIS METHODS

The proportion of surviving larvae, and the proportion of normal surviving larvae were calculated for each treatment replicate. The calculation used for the proportion of normal surviving larvae, Combined Proportion Normal, was the combined endpoint specified by EPA/600/R-95/136. The means were obtained for each treatment level and the latter were then corrected for control response using Abbott's formula. The LC50 (survival) and the EC50 (normality) were calculated, where data permitted, using either the Maximum-Likelihood Probit or the Trimmed Spearman-Karber methods. An IC25 was determined by linear interpolation with bootstrapping. NOEC and LOEC values for survival and normality were computed using either Dunnett's test, T-test with Bonferroni's adjustment, Steel's Many-One Rank Test, or Wilcoxon Rank Sum Test with Bonferroni Adjustment. The appropriate test was selected after evaluating the data for normality and homogeneity of variance. An arcsine-square root (angular) transformation was performed on the data prior to statistical analysis. The statistical software employed for these calculations was CETIS, v1.7.0C, Tidepool Scientific Software. Toxic units (TU_c) were computed as 100/NOEC, 100/EC50, or 100/IC25.

PROTOCOL DEVIATIONS

None

REFERENCE TOXICANT TEST

The routine reference toxicant test is a standard multi-concentration toxicity test using copper sulfate to evaluate the performance of the test organisms used in the effluent toxicity test. The performance is evaluated by comparing the results of this test with historical results obtained at the laboratory. A summary of the reference toxicant test result is given below. The reference toxicant test raw data are found in Appendix III.

Test No.: 999-2595
Reference Toxicant and Source: Copper as $CuSO_4 \cdot 5H_2O$, Argent Lot No. 0195. Concentrated stock prepared 8-3-07.
Test Date: 7-22-09
Dilution Water Used: Yaquina Bay, OR seawater. Salinity 30.0 ‰, pH 8.1.

Results: EC50, 10.9 µg/L; NOEC, 8 µg/L; IC25, 9.42 µg/L. The EC50 results are within the laboratory's control chart warning limits (8.02 – 12.1 µg/L).

TEST RESULTS

Detailed tabulations of the test results are given in Table 1. The biological effects, given as the NOEC, LOEC, EC50/LC50 for normality and survival, and IC25 for normality are summarized below.

	Combined Proportion Normal	Survival
NOEC (%)	0.16 (TU_c=625)	0.16 (TU_c=625)
LOEC (%)	>0.16 (TU_c<625)	>0.16 (TU_c<625)
EC50/LC50 (%)	>0.16 (TU_c<625)	>0.16 (TU_c<625)
(95% C.I.)	—	—
Method of Calculation	By Data Inspection	By Data Inspection
IC25 (%)	>0.16 (TU_c<625)	
(95% C.I.)	—	
Method of Calculation	Linear Interpolation	

DISCUSSION/CONCLUSIONS

The NOEC was 0.16 % effluent, and the EC50 and IC25 for abnormal development were both >0.16 %.

STUDY APPROVAL

Project Manager — Date: 8-21-09

Study Director — Date: 8-21-09

Laboratory Director — Date: 8/21/09

Quality Assurance Unit — Date: 8/19/09

Table 1. Test response of mussel (*Mytilus galloprovincialis*) larvae exposed to XTO Energy – Platform A – water flood.

Test Material					Combined Proportion Normal*		Proportion Survived*	
Concentration (%)	Repl.	Norm.	Abn.	Total		Mean		Mean
0.16	1	244	2	246	0.897		0.904	
	2	231	3	234	0.849		0.860	
	3	245	4	249	0.901		0.915	
	4	271	10	281	0.996	0.911	1.000	0.920
0.08	1	258	9	267	0.949		0.982	
	2	243	7	250	0.893		0.919	
	3	210	6	216	0.772		0.794	
	4	242	7	249	0.890	0.876	0.915	0.903
0.04	1	262	4	266	0.963		0.978	
	2	267	4	271	0.982		0.996	
	3	246	11	257	0.904		0.945	
	4	247	6	253	0.908	0.939	0.930	0.962
0.02	1	263	6	269	0.967		0.989	
	2	270	7	277	0993		1.000	
	3	287	8	295	0.973		1.000	
	4	268	6	274	0.985	0.979	1.000	0.997
0.01	1	286	5	291	0.983		1.000	
	2	268	10	278	0.985		1.000	
	3	231	4	235	0.849		0.864	
	4	238	9	247	0.875	0.923	0.908	0.943
Normal Control	1	260	5	265	0.956		0.974	
	2	278	8	286	0.972		1.000	
	3	254	11	265	0.934		0.974	
	4	229	5	234	0.842	0.926	0.860	0.952

* Based on an average initial count of 272 embryos per 10 ml sample, except that for the case in the combined proportion normal endpoint where number normal>average initial count, number normal is divided by the total count (as per EPA/600/R-95/136).

† Result significantly different (P≤0.05) from the control.

TOXICITY TEST REPORT

TEST IDENTIFICATION

Test No.: 663-65

Title: Mussel (*Mytilus galloprovincialis*) larval test using static 48-hr exposure to XTO Energy – Platform C – non-contact cooling water.

Protocol No.: NAS-XXX-CG/MG2, August 28, 1990, Revision 3 (9-8-01). This protocol complies with the U.S. EPA West Coast chronic toxicity manual (EPA/600/R-95/136) and ASTM bivalve toxicity method (E 724-89).

STUDY MANAGEMENT

Study Sponsor: XTO Energy, 52260 Wik Rd, Kenai, AK 99611

Sponsor's Study Monitor: Mr. Ryan Tunseth

Testing Laboratory: Northwestern Aquatic Sciences, P.O. Box 1437, Newport, OR 97365.

Test Location: Newport laboratory.

Laboratory's Study Personnel: G.A. Buhler, B.S., Proj. Man.; G.J. Irissarri, B.S., Study Dir.; L.K. Nemeth, B.A., M.B.A., QA Officer; M.S. Redmond, M.S., Aq. Toxicol.; S.J. Gage, B.A., Sr. Tech.

Study Schedule:

Test Beginning: 7-22-09, 1445 hrs.

Test Ending: 7-24-09, 1450 hrs.

Disposition of Study Records: All specimens, raw data, reports and other study records are stored according to Good Laboratory Practice regulations at Northwestern Aquatic Sciences, 3814 Yaquina Bay Rd., Newport, OR 97365.

Good Laboratory Practices: The test was conducted following the principles of Good Laboratory Practices (GLP) as defined in the EPA/TSCA Good Laboratory Practice regulations revised August 17, 1989 (40 CFR Part 792).

Statement of Quality Assurance: The test data were reviewed by the Quality Assurance Unit to assure that the study was performed in accordance with the protocol and standard operating procedures. This report is an accurate reflection of the raw data.

TEST MATERIAL

Description: XTO Energy – Platform C – non-contact cooling water. Details are as follows:

NAS Sample No.	2801G
Collection Date	7-21-09
Receipt Date	7-22-09
Temperature (°C)	5.1
pH	7.9
Dissolved oxygen (mg/L)	10.5
Salinity (‰)	27.0

Treatments: Sample was briefly temperature-equilibrated prior to use.

Storage: Used date of receipt.

DILUTION WATER

Source: Yaquina Bay, Oregon.

Date of Collection: 7-21-09

Water Quality: Salinity, 30.0 ‰; pH, 8.1

Pretreatment: Filtered to 0.4 µm, aerated, salinity adjusted with Milli-Q water.

BRINE USED FOR SALINITY CONTROL

None Used

TEST ORGANISMS

Species: Mussel (*Mytilus galloprovincialis*).
Age: 2.0 hours post-fertilization.
Source: Carlsbad Aquafarm, Carlsbad, CA.
Conditioning: Adult mussels were received on 7-17-09 and placed in trays with flowing seawater. Holding conditions for the five days prior to the test averaged: temperature, 16.8 ± 1.4°C; pH, 7.3 ± 0.6; salinity, 33.8 ± 0.3 ‰; and dissolved oxygen, 5.6 ± 0.8 mg/L. Photoperiod was natural daylight.
Source of Gametes: 1 female and 1 male.

TEST PROCEDURES AND CONDITIONS

Test Chambers: 30 ml borosilicate glass vials containing 10 ml of test solutions.
Test Concentrations: 0.16, 0.08, 0.04, 0.02, 0.01, and 0% (Control).
Brine Control: None used
Replicates/Treatment: 4
Initial Concentration of Test Organisms: 27.2/ml.
Volume of Subsamples Taken for Counting: NA
Water Volume Changes per 24 hr: None (non-renewal static test).
Aeration: None
Feeding: None
Effects Criteria: The effect criteria used were: 1) ability of embryos to survive and produce completely developed shells; and 2) survival. Data collected were: 1) the initial embryo density; 2) the number of abnormal larvae observed; and 3) the number of normal (live with completely developed shells) larvae observed.
Water Quality and Other Test Conditions: Temperature, 15.7 ± 0.3°C; pH, 8.1 ± 0.0; salinity, 30.0 ± 0.3 ‰; and dissolved oxygen, 8.1 ± 0.0 mg/L. Photoperiod 16:8 hr, L:D.

DATA ANALYSIS METHODS

The proportion of surviving larvae, and the proportion of normal surviving larvae were calculated for each treatment replicate. The calculation used for the proportion of normal surviving larvae, Combined Proportion Normal, was the combined endpoint specified by EPA/600/R-95/136. The means were obtained for each treatment level and the latter were then corrected for control response using Abbott's formula. The LC50 (survival) and the EC50 (normality) were calculated, where data permitted, using either the Maximum-Likelihood Probit or the Trimmed Spearman-Karber methods. An IC25 was determined by linear interpolation with bootstrapping. NOEC and LOEC values for survival and normality were computed using either Dunnett's test, T-test with Bonferroni's adjustment, Steel's Many-One Rank Test, or Wilcoxon Rank Sum Test with Bonferroni Adjustment. The appropriate test was selected after evaluating the data for normality and homogeneity of variance. An arcsine-square root (angular) transformation was performed on the data prior to statistical analysis. The statistical software employed for these calculations was CETIS, v1.7.0C, Tidepool Scientific Software. Toxic units (TU_c) were computed as 100/NOEC, 100/EC50, or 100/IC25.

PROTOCOL DEVIATIONS

None

REFERENCE TOXICANT TEST

The routine reference toxicant test is a standard multi-concentration toxicity test using copper sulfate to evaluate the performance of the test organisms used in the effluent toxicity test. The performance is evaluated by comparing the results of this test with historical results obtained at the laboratory. A summary of the reference toxicant test result is given below. The reference toxicant test raw data are found in Appendix III.

Test No.: 999-2595
Reference Toxicant and Source: Copper as $CuSO_4 \cdot 5H_2O$, Argent Lot No. 0195. Concentrated stock prepared 8-3-07.
Test Date: 7-22-09
Dilution Water Used: Yaquina Bay, OR seawater. Salinity 30.0 ‰, pH 8.1.

Results: EC50, 10.9 µg/L; NOEC, 8 µg/L; IC25, 9.42 µg/L. The EC50 results are within the laboratory's control chart warning limits (8.02 – 12.1 µg/L).

TEST RESULTS

Detailed tabulations of the test results are given in Table 1. The biological effects, given as the NOEC, LOEC, EC50/LC50 for normality and survival, and IC25 for normality are summarized below.

	Combined Proportion Normal	Survival
NOEC (%)	0.16 (TU_c=625)	0.16 (TU_c=625)
LOEC (%)	>0.16 (TU_c<625)	>0.16 (TU_c<625)
EC50/LC50 (%)	>0.16 (TU_c<625)	>0.16 (TU_c<625)
(95% C.I.)	---	---
Method of Calculation	By Data Inspection	By Data Inspection
IC25 (%)	>0.16 (TU_c<625)	
(95% C.I.)	---	
Method of Calculation	Linear Interpolation	

DISCUSSION/CONCLUSIONS

The NOEC was 0.16 % effluent, and the EC50 and IC25 for abnormal development were both >0.16 %.

STUDY APPROVAL

Project Manager — Date 8-21-09

Study Director — Date 8-21-09

Laboratory Director — Date 8/21/09

Quality Assurance Unit — Date 8/19/09

Table 1. Test response of mussel (*Mytilus galloprovincialis*) larvae exposed to XTO Energy – Platform C – non-contact cooling water.

Test Material					Combined Proportion Normal*		Proportion Survived*	
Concentration (%)	Repl.	Norm.	Abn.	Total		Mean		Mean
0.16	1	237	8	245	0.871		0.901	
	2	228	7	235	0.838		0.864	
	3	285	11	296	0.963		1.000	
	4	259	3	262	0.952	0.906	0.963	0.932
0.08	1	246	7	253	0.904		0.930	
	2	258	6	264	0.949		0.971	
	3	244	5	249	0.897		0.915	
	4	263	6	269	0.967	0.929	0.989	0.951
0.04	1	260	8	268	0.956		0.985	
	2	220	12	232	0.809		0.853	
	3	240	7	247	0.882		0.908	
	4	226	8	234	0.831	0.870	0.860	0.902
0.02	1	268	8	276	0.985		1.000	
	2	263	3	266	0.967		0.978	
	3	260	10	270	0.956		0.993	
	4	249	8	257	0.915	0.956	0.945	0.979
0.01	1	261	11	272	0.960		1.000	
	2	268	4	272	0.985		1.000	
	3	228	6	234	0.838		0.860	
	4	252	9	261	0.927	0.927	0.960	0.955
Normal Control	1	264	2	266	0.971		0.978	
	2	224	5	229	0.824		0.842	
	3	259	5	264	0.952		0.971	
	4	249	7	256	0.915	0.915	0.941	0.933

* Based on an average initial count of 272 embryos per 10 ml sample, except that for the case in the combined proportion normal endpoint where number normal>average initial count, number normal is divided by the total count (as per EPA/600/R-95/136).

† Result significantly different (P≤0.05) from the control.

TOXICITY TEST REPORT

TEST IDENTIFICATION

Test No.: 663-64

Title: Mussel (*Mytilus galloprovincialis*) larval test using static 48-hr exposure to XTO Energy – Platform C – water flood.

Protocol No.: NAS-XXX-CG/MG2, August 28, 1990, Revision 3 (9-8-01). This protocol complies with the U.S. EPA West Coast chronic toxicity manual (EPA/600/R-95/136) and ASTM bivalve toxicity method (E 724-89).

STUDY MANAGEMENT

Study Sponsor: XTO Energy, 52260 Wik Rd, Kenai, AK 99611

Sponsor's Study Monitor: Mr. Ryan Tunseth

Testing Laboratory: Northwestern Aquatic Sciences, P.O. Box 1437, Newport, OR 97365.

Test Location: Newport laboratory.

Laboratory's Study Personnel: G.A. Buhler, B.S., Proj. Man.; G.J. Irissarri, B.S., Study Dir.; L.K. Nemeth, B.A., M.B.A., QA Officer; M.S. Redmond, M.S., Aq. Toxicol.; S.J. Gage, B.A., Sr. Tech.

Study Schedule:

Test Beginning: 7-22-09, 1445 hrs.

Test Ending: 7-24-09, 1450 hrs.

Disposition of Study Records: All specimens, raw data, reports and other study records are stored according to Good Laboratory Practice regulations at Northwestern Aquatic Sciences, 3814 Yaquina Bay Rd., Newport, OR 97365.

Good Laboratory Practices: The test was conducted following the principles of Good Laboratory Practices (GLP) as defined in the EPA/TSCA Good Laboratory Practice regulations revised August 17, 1989 (40 CFR Part 792).

Statement of Quality Assurance: The test data were reviewed by the Quality Assurance Unit to assure that the study was performed in accordance with the protocol and standard operating procedures. This report is an accurate reflection of the raw data.

TEST MATERIAL

Description: XTO Energy Platform C – water flood. Details are as follows:

NAS Sample No.	2800G
Collection Date	7-21-09
Receipt Date	7-22-09
Temperature (°C)	5.2
pH	8.0
Dissolved oxygen (mg/L)	10.5
Salinity (‰)	27.0

Treatments: Sample was briefly temperature-equilibrated prior to use.

Storage: Used date of receipt.

DILUTION WATER

Source: Yaquina Bay, Oregon.

Date of Collection: 7-21-09

Water Quality: Salinity, 30.0 ‰; pH, 8.1

Pretreatment: Filtered to 0.4 µm, aerated, salinity adjusted with Milli-Q water.

BRINE USED FOR SALINITY CONTROL

None Used

TEST ORGANISMS

Species: Mussel (*Mytilus galloprovincialis*).
Age: 2.0 hours post-fertilization.
Source: Carlsbad Aquafarm, Carlsbad, CA.
Conditioning: Adult mussels were received on 7-17-09 and placed in trays with flowing seawater. Holding conditions for the five days prior to the test averaged: temperature, 16.8 ± 1.4°C; pH, 7.3 ± 0.6; salinity, 33.8 ± 0.3 ‰; and dissolved oxygen, 5.6 ± 0.8 mg/L. Photoperiod was natural daylight.
Source of Gametes: 1 female and 1 male.

TEST PROCEDURES AND CONDITIONS

Test Chambers: 30 ml borosilicate glass vials containing 10 ml of test solutions.
Test Concentrations: 0.16, 0.08, 0.04, 0.02, 0.01, and 0% (Control).
Brine Control: None used
Replicates/Treatment: 4
Initial Concentration of Test Organisms: 27.2/ml.
Volume of Subsamples Taken for Counting: NA
Water Volume Changes per 24 hr: None (non-renewal static test).
Aeration: None
Feeding: None
Effects Criteria: The effect criteria used were: 1) ability of embryos to survive and produce completely developed shells; and 2) survival. Data collected were: 1) the initial embryo density; 2) the number of abnormal larvae observed; and 3) the number of normal (live with completely developed shells) larvae observed.
Water Quality and Other Test Conditions: Temperature, 15.6 ± 0.3°C; pH, 8.1 ± 0.0; salinity, 29.8 ± 0.2 ‰; and dissolved oxygen, 8.1 ± 0.1 mg/L. Photoperiod 16:8 hr, L:D.

DATA ANALYSIS METHODS

The proportion of surviving larvae, and the proportion of normal surviving larvae were calculated for each treatment replicate. The calculation used for the proportion of normal surviving larvae, Combined Proportion Normal, was the combined endpoint specified by EPA/600/R-95/136. The means were obtained for each treatment level and the latter were then corrected for control response using Abbott's formula. The LC50 (survival) and the EC50 (normality) were calculated, where data permitted, using either the Maximum-Likelihood Probit or the Trimmed Spearman-Karber methods. An IC25 was determined by linear interpolation with bootstrapping. NOEC and LOEC values for survival and normality were computed using either Dunnett's test, T-test with Bonferroni's adjustment, Steel's Many-One Rank Test, or Wilcoxon Rank Sum Test with Bonferroni Adjustment. The appropriate test was selected after evaluating the data for normality and homogeneity of variance. An arcsine-square root (angular) transformation was performed on the data prior to statistical analysis. The statistical software employed for these calculations was CETIS, v1.7.0C, Tidepool Scientific Software. Toxic units (TU_c) were computed as 100/NOEC, 100/EC50, or 100/IC25.

PROTOCOL DEVIATIONS

None

REFERENCE TOXICANT TEST

The routine reference toxicant test is a standard multi-concentration toxicity test using copper sulfate to evaluate the performance of the test organisms used in the effluent toxicity test. The performance is evaluated by comparing the results of this test with historical results obtained at the laboratory. A summary of the reference toxicant test result is given below. The reference toxicant test raw data are found in Appendix III.

Test No.: 999-2595
Reference Toxicant and Source: Copper as $CuSO_4 \cdot 5H_2O$, Argent Lot No. 0195. Concentrated stock prepared 8-3-07.
Test Date: 7-22-09
Dilution Water Used: Yaquina Bay, OR seawater. Salinity 30.0 ‰, pH 8.1.

Results: EC50, 10.9 µg/L; NOEC, 8 µg/L; IC25, 9.42 µg/L. The EC50 results are within the laboratory's control chart warning limits (8.02 – 12.1 µg/L).

TEST RESULTS

Detailed tabulations of the test results are given in Table 1. The biological effects, given as the NOEC, LOEC, EC50/LC50 for normality and survival, and IC25 for normality are summarized below.

	Combined Proportion Normal	Survival
NOEC (%)	0.16 (TU_c=625)	0.16 (TU_c=625)
LOEC (%)	>0.16 (TU_c<625)	>0.16 (TU_c<625)
EC50/LC50 (%)	>0.16 (TU_c<625)	>0.16 (TU_c<625)
(95% C.I.)	---	---
Method of Calculation	By Data Inspection	By Data Inspection
IC25 (%)	>0.16 (TU_c<625)	
(95% C.I.)	---	
Method of Calculation	Linear Interpolation	

DISCUSSION/CONCLUSIONS

The NOEC was 0.16 % effluent, and the EC50 and IC25 for abnormal development were both >0.16 %.

STUDY APPROVAL

Gary Burbach 9-21-09
Project Manager Date

Gerald Quiram 8-21-09
Study Director Date

Richard A. Caldwell 8/21/09
Laboratory Director Date

Lynette K. Nemeth 8/19/09
Quality Assurance Unit Date

Table 1. Test response of mussel (*Mytilus galloprovincialis*) larvae exposed to XTO Energy Platform C – water flood.

Test Material					Combined Proportion Normal*		Proportion Survived*	
Concentration (%)	Repl.	Norm.	Abn.	Total		Mean		Mean
0.16	1	283	9	292	0.969		1.000	
	2	245	7	252	0.901		0.927	
	3	267	4	271	0.982		0.996	
	4	251	3	254	0.923	0.944	0.934	0.964
0.08	1	263	12	275	0.967		1.000	
	2	237	8	245	0.871		0.901	
	3	245	4	249	0.901		0.915	
	4	245	4	249	0.901	0.910	0.915	0.933
0.04	1	250	9	259	0.919		0.952	
	2	242	9	251	0.890		0.923	
	3	265	7	272	0.974		1.000	
	4	247	4	251	0.908	0.923	0.923	0.949
0.02	1	246	8	254	0.904		0.934	
	2	285	9	294	0.969		1.000	
	3	237	9	246	0.871		0.904	
	4	274	8	282	0.972	0.929	1.000	0.960
0.01	1	276	4	280	0.986		1.000	
	2	262	3	265	0.963		0.974	
	3	232	3	235	0.853		0.864	
	4	247	6	253	0.908	0.928	0.930	0.942
Normal Control	1	223	7	230	0.820		0.846	
	2	268	6	274	0.985		1.000	
	3	242	6	248	0.890		0.912	
	4	258	10	268	0.949	0.911	0.985	0.936

* Based on an average initial count of 272 embryos per 10 ml sample, except that for the case in the combined proportion normal endpoint where number normal>average initial count, number normal is divided by the total count (as per EPA/600/R-95/136).

† Result significantly different (P≤0.05) from the control.